Supplemental Figures

Figure S1, related to Figure 2

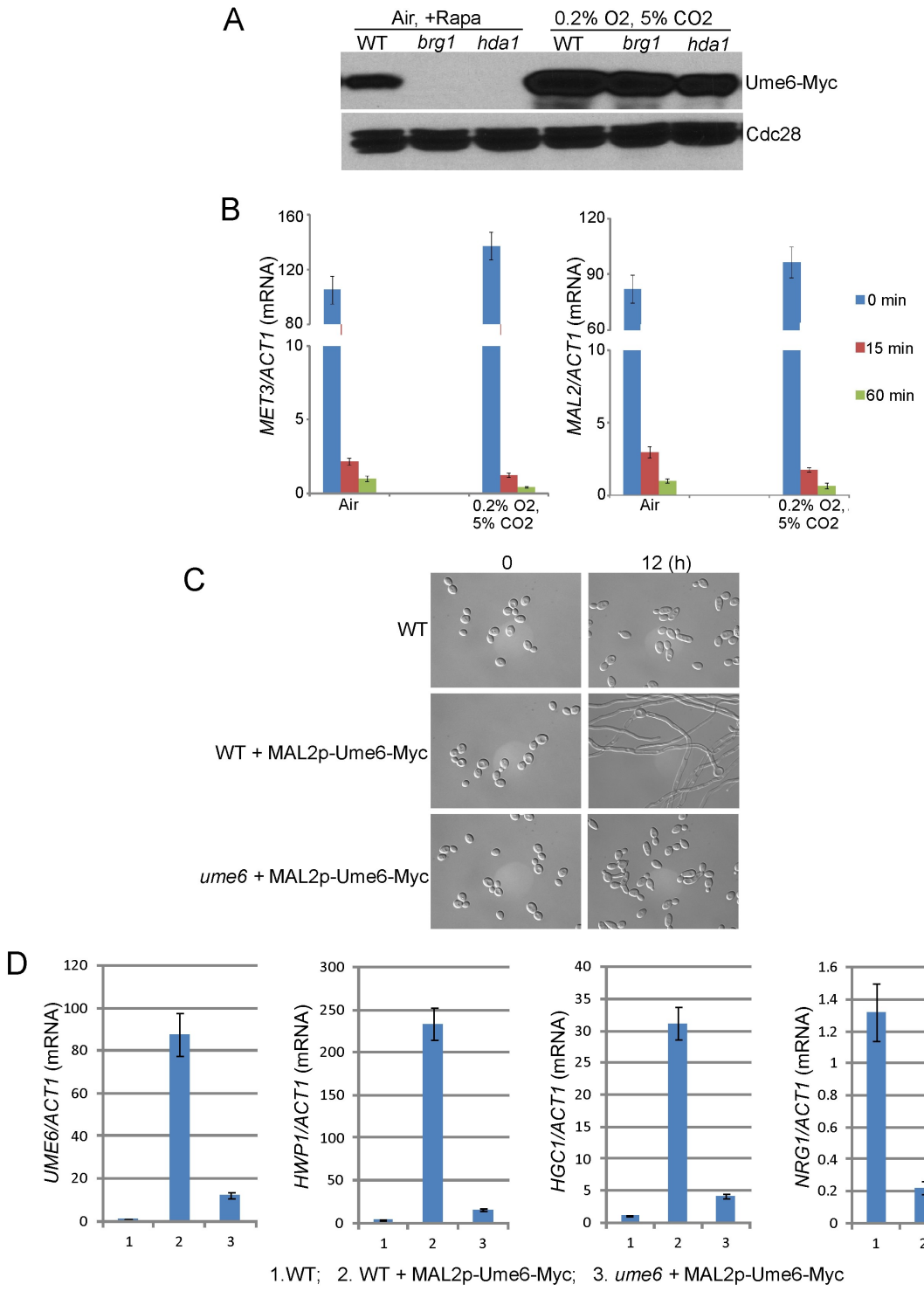

Figure S1. The protein levels of Ume6 are extremely high in hypoxia plus 5% CO2 during hyphal elongation, and ectopically expressed Ume6 is sufficient for sustained hyphal development and hypha-specific transcription.

(A). Wild-type cells carrying Ume6-Myc were diluted into YPSucose media under indicated conditions, and cells were collected at 8 h for Western analysis.

(B). Promoter shutdown of *MET3* and *MAL2* in hypoxia with 5% CO2 is similar to that in normoxia. *MET3* and *MAL2* mRNA levels were determined by qRT-PCR. The signal obtained from *ACT1* mRNA was used for normalization. The 60 min values in normoxia were set to be 1.00. Mean data ± SEM from three independent qRT-PCR experiments are plotted.

(C). Wild type or *ume6* mutant cells carrying Ume6-Myc under the *MAL2* promoter were diluted 1:100 fold into YEPMaltose medium at 37°C, and cells were collected at 0 h or 12 h for cell morphology. Wild-type cells transformed with pBES116 were used as control strains.

(D). mRNA levels were measured by qRT-PCR experiments in the same strains under the same growth conditions shown in Figure S1C. The signal obtained from *ACT1* mRNA was used as a loading control for normalization. Mean data ± SEM from three independent qRT-PCR experiments are plotted.

Figure S2, related to Figure 3

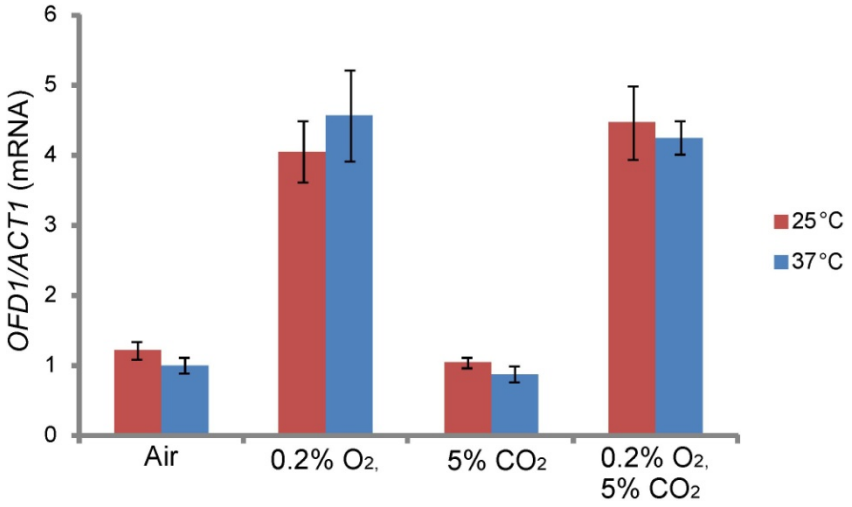

Figure S2. The expression levels of *OFD1* increase under hypoxic condition.

Wild-type cells were incubated under indicated conditions for 6 h. *OFD1* expression levels were quantified by qRT-PCR and normalized with *ACT1*. The value in normoxia at 37^{o}C was set to be 1.00. Mean data ± SEM from three independent qRT-PCR experiments are plotted.

Supplemental Tables

Table S 1. *C. albicans* stains used in this study

Strain	Parent / background	Genotype	Source
SC5314		Wild type	(Fonzi and Irwin, 1993)
CAI4	SC5314	*ura3::1 imm434/ura3::1 imm434*	(Fonzi and Irwin, 1993)
BWP17	SC5314	*ura3*::*1 imm434/ura3*::*1 imm434 his1*::*hisG/his1*::*hisG arg4*::*hisG/arg4*::*hisG*	(Wilson et al., 1999)
DAY185	BWP17	*ura3*::λ*imm434/ura3*::λ*imm434 HIS1*::*his1*::*hisG/his1*::*hisG ARG4*::*URA3*::*arg4*::*hisG/arg4*::*hisG*	(Davis et al., 2000)
UZ43	UZ24	*leu2/leu2 his1/his1 URA3/ura3 ume6::HIS1/ume6::LEU2*	(Zeidler et al., 2009)
HLY4176	CAI4	*ura3*::*1 imm434/ura3*::*1 imm434 MET3/met3*::*MET3p-UME6*$_{C778/785S}$*-13MYC-URA3*	This study
HLY3578	BWP17	*ura3*::*1 imm434/ura3*::*1 imm434 his1*::*hisG/his1*::*hisG arg4*::*hisG/arg4*::*hisG ADE2/ade2*::*MAL2p-MYC-HGC1-URA3*	(Wang et al., 2007)
HLY4113	BWP17	*ofd1*::*ARG4/ofd1*::*URA3 /ofd1*::*HIS1 ura3*::*1 imm434/ura3*::*1 imm434 his1*::*hisG/his1*::*hisG arg4*::*hisG/arg4*::*hisG*	This study
HLY4078	CAI4	*ura3::1 imm434/ura3::1 imm434 UME6/UME6-13MYC-URA3*	(Lu et al., 2012)
HLY4114	HLY4113	*ofd1*::*ARG4/ofd1*::*URA3 /ofd1*::*HIS1 ura3*::*1 imm434/ura3*::*1 imm434 his1*::*hisG/his1*::*hisG arg4*::*hisG/arg4*::*hisG RP10::ACT1p-OFD1-13MYC-SAT1*	This study
HLY4115	HLY4113	*ofd1*::*ARG4/ofd1*::*URA3 /ofd1*::*HIS1 ura3*::*1 imm434/ura3*::*1 imm434 his1*::*hisG/his1*::*hisG arg4*::*hisG/arg4*::*hisG RP10::ACT1p-OFD1*$_{1-260}$*-13MYC-SAT1*	This study
HLY4116		*ofd1*::*ARG4/ofd1*::*URA3 /ofd1*::*HIS1 ura3*::*1 imm434/ura3*::*1 imm434 his1*::*hisG/his1*::*hisG arg4*::*hisG/arg4*::*hisG RP10::ACT1p-OFD1*$_{261-617}$*-13MYC-SAT1*	This study
HLY4117		*ofd1*::*ARG4/ofd1*::*URA3 /ofd1*::*HIS1 ura3*::*1 imm434/ura3*::*1 imm434 his1*::*hisG/his1*::*hisG arg4*::*hisG/arg4*::*hisG RP10::ACT1p-OFD1*$^{H162A/D164A}$*-13MYC-SAT1*	This study
HLY 4035	CLY4	*ura3*::*1 imm434/ura3*::*1 imm434 yng2*::*hisG/YNG2-13MYC -URA3*	(Lu et al., 2011)
HLY4036	CLY4	*ura3*::*1 imm434/ura3*::*1 imm434 yng2*::*hisG/YNG2*^{K175R}*-13MYC -URA3*	(Lu et al., 2011)
HLY4118		*ura3*::*1 imm434/ura3*::*1 imm434 yng2*::*hisG/YNG2-13MYC -URA3 RP10::ACT1p- OFD1*$_{261-617}$*-13MYC-SAT1*	This study
HLY4037	CLY4	*ura3*::*1 imm434/ura3*::*1 imm434 yng2*::*hisG/YNG2*^{K175Q}*-13MYC -URA3*	(Lu et al., 2011)
HLY4119	HLY4037	*ura3*::*1 imm434/ura3*::*1 imm434 yng2*::*hisG/YNG2*^{K175Q}*-13MYC -URA3 RP10::ACT1p- OFD1*$_{261-617}$*-13MYC-SAT1*	This study

Table S 2. Primers used in this study

Primer	Sequnce	Purpose and feachers
1	5’- GTCAGGCCTCAATTAGAGGTTGTGGATGC	MET3 promoter
2	5’- CGGGATCCGTTTTCTGGGGAGGGTATTT	
3	5’- CTGGGATCCATGATTACCCATATGGTTAC	pMET3-UME6$^{C778/785S}$-MYC
4	5’- CAGAAATTGGTGTGACTTCATCAGATTTCAAATGTTTAATTCGACAA	
5	5’- ATCTGATGAAGTCACACCAATTTCTGGAGGTTGTGCTAAATTTGGA	
6	5’- GGCGACGCGTCGATCATTGGTTATATCATTAC	
7	5’-GTACCTCGAGGTCGAG CGTCAAAACTAGAG	pPR671-SAT1
8	5’CACTTGCAGGTCTGGTTGTTCGGAACCCTCAGTTTCATTTGCCACA CCAAC AGTTTGATGAAAGTCTCTGCAG GACCACCTTTGATTG	
9	5’- CGGGATCCC ATGACACCTAGTAAAAGAAG	pPR671-OFD1-SAT1
10	5’-GGCGACGCGTCG ATTTTCATTACCCTCTTCTT	
11	5’- GGCGACGCGTCG AGTTTCTTCTTGTTCACCAG	pPR671-OFD1$_{1-260}$-SAT1
12	5’- CGGGATCCCATGGAGGCAAGATCAACATTACA	pPR671-OFD1$_{261-617}$-SAT1
13	5’- AATGACAGCATCAGCAGTCAATAAATGACATCCTTTTCTA	pPR671-OFD1$^{H162A/D164A}$-SAT1
14	5’- TGACTGCTGATGCTGTCATTGGTAGTAGAAGAGTTAGTTT	

Restriction sites are underlined.

Supplemental Experimental Procedures

Plasmid and strain construction

The *C. albicans* strains used in this study are listed in Table S1. Primer sequences are listed in Table S2. *OFD1* was deleted based on the method as described previously(Wilson et al., 1999). The disruption was confirmed by PCR (data not shown). The *MET3* promoter sequence was amplified using primers 1 and 2. The resulting 1.6-kb PCR product was digested with StuI and BamHI and inserted into the StuI-BamHI site of pPR673(Lu et al., 2008) to create pPR673-MET3p. Two-step PCR was used to create pMET3-UME6$^{C778/785S}$-13MYC. Two pairs of primers (primers 3 and 4, 5 and 6) were used to PCR amplify overlapping *UME6* fragments with the mutation in the overlapping region. The resulting PCR products were purified and mixed as templates for another round of PCR amplification using the primers 3 and 6, which produced the full-length *UME6$^{C778/785S}$* sequence. The resulting mutant, *UME6$^{C778/785S}$*, was inserted into the BamHI-MluI sites of pPR673-MET3p to generate pMET3-UME6$^{C778/785S}$-MYC. The plasmid was digested with PmlI within the *MET3* promoter region for integration into the endogenous *MET3* locus. The pPR671-SAT1 plasmid was constructed by amplifying the *SAT1* cassette (primers 7 and 8) from pSFS2 plasmid(Reuss et al., 2004). The PCR product was digested with *Xho*I and *Pst*I to replace HIS1 marker from pPR671 plasmid(Cao et al., 2006). The *OFD1* coding sequence was amplified using primers 9 and 10. The resulting 2.0-kb PCR product was digested with BamHI and MluI and inserted into the BamHI-MluI site of pPR671-SAT1 to create pACT1-OFD1-13MYC. A 0.8-kb PCR product (primers 9 and 11) containing the N-terminal *OFD1* coding region and a 1.2-kb PCR product (primers 10 and 12) containing the C-terminal *OFD1*coding

region were inserted into the BamHI-MluI sites of pPR671-SAT1. Two-step PCR was used to create pACT1-OFD1$^{H162A/D164A}$-13MYC. Two pairs of primers (primers 9 and 13, 10 and 14) were used to PCR amplify overlapping *OFD1* fragments with the mutation in the overlapping region. The resulting PCR products were purified and mixed as templates for another round of PCR amplification using the primers 7 and 8, which produced the full-length *OFD1$^{H162A/D164A}$* sequence. The resulting mutant, *OFD1$^{H162A/D164A}$*, was cloned into the BamHI-MluI site of the plasmid pACT1-OFD1-13MYC, replacing the wild-type copy, and was confirmed by DNA sequencing. These plasmids were digested with StuI to target integration into *RP10* locus.

Chromatin Immunoprecipitation

Chromatin immunoprecipitation was performed as described with modifications (Lu et al., 2008). DNA was sheared by sonication six times for 20 seconds at high power on a Bioruptor (diagenode) with 40 seconds intervals on ice. 10 µl of anti-Myc (SC-789, Santa Cruz) antibodies were used for ~ 4 mg of chromatin proteins in an immunoprecipitation volume of 200 µl.

Quantitative PCR Expression Analysis

Methods for RNA isolation were carried out as previously described(Lane et al., 2001). 10 µg of total RNA was DNase-treated at 37°C for 1 h using the DNase-free kit (Qiagen), cDNA was synthesized using the SuperScript II Reverse Transcriptase kit (Invitrogen), and qPCR was done using the iQ SYBR Green Supermix (Bio-Rad).

Supplemental References

Davis, D., Edwards, J. E., Jr., Mitchell, A. P., and Ibrahim, A. S. (2000). Candida albicans RIM101 pH response pathway is required for host-pathogen interactions. Infect Immun *68*, 5953-5959.
Fonzi, W. A., and Irwin, M. Y. (1993). Isogenic strain construction and gene mapping in Candida albicans. Genetics *134*, 717-728.
Lane, S., Birse, C., Zhou, S., Matson, R., and Liu, H. (2001). DNA array studies demonstrate convergent regulation of virulence factors by Cph1, Cph2, and Efg1 in Candida albicans. J Biol Chem *276*, 48988-48996.
Lu, Y., Su, C., Mao, X., Raniga, P. P., Liu, H., and Chen, J. (2008). Efg1-mediated recruitment of NuA4 to promoters is required for hypha-specific Swi/Snf binding and activation in Candida albicans. Mol Biol Cell *19*, 4260-4272.
Reuss, O., Vik, A., Kolter, R., and Morschhauser, J. (2004). The SAT1 flipper, an optimized tool for gene disruption in Candida albicans. Gene *341*, 119-127.
Wilson, R. B., Davis, D., andMitchell, A. P. (1999). Rapid hypothesis testing with Candida albicans through gene disruption with short homology regions. J Bacteriol *181*, 1868-1874.